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OVERWINTERING OF CERTAIN WHEAT PATHOGENES

William Robert Foster

Department of Field Crops

University of Alberta
Edmonton, Alberta
April, 1930

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OVERWINTERING OF CERTAIN WHEAT PATHOGENES

William Robert Foster
Department of Field Crops

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements for
the degree of

MASTER OF SCIENCE.

Edmonton, Alberta.

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OVERWINTERING OF CERTAIN WHEAT PATHOGENES

By W. R. Foster.

Introduction.

It is important to know in what form and under what conditions plant pathogenic organisms overwinter. We have relatively little specific information of this sort for pathogenes affecting crop plants in western Canada, a region in which the winters are probably as severe as in any other large wheat growing area of the world.

Plant pathogenes may overwinter in vegetative or reproductive stages or both. The vegetative stage in parasitic fungi consists of a mycelium which may be either external or internal; that is, it may develop on the surface or within the tissues of the host. Although the vegetative mycelium is capable of reproducing the fungus, fruiting organs are usually formed. In many of the fungi, more than one type of spore is developed. If the pathogenes are capable of living over our severe winters it is important to know what stage or stages survive.

The main factors which affect the ability of the organisms to survive are the medium in which they live, and the environment. Plant pathogenes vary greatly in their

ability to live on different substrata. Some organisms require living tissue and are called obligate parasites; some normally pass through life as parasites but are capable, in certain stages, of a true saprophytic existence, and there are still others which are facultative parasites. Each of these may assume dormant conditions in various stages. Plant pathogenes may overwinter in or on the seed, the plant, the soil and the stubble or other dead organic material. The character of the substratum and the position of the inoculum on or in it, might greatly affect survival. Furthermore, some substrata may contain toxic or inhibitory substances while others may not. Thus, Kotila and Coons (26), in their overwintering studies of the blackleg organism, attribute its disappearance to a large extent to a lytic principle which they have isolated from the soil, water and the plant.

Environmental factors vary greatly in the different wheat growing areas of the world. They fluctuate widely from season to season. These factors may have a pronounced effect on the pathogenes which attack plants. Temperature for instance plays a very important part in the germination of spores. It may also directly affect the growth and survival of the vegetative parts of a pathogene. Often, the survival of a fungus is imperilled by the weather conditions of the previous summer and autumn which may prevent the formation of the very spores by which it normally survives the winter; or

such spores may be actually killed. According to Foister (13), a hot dry period in 1921 killed all the urediniospores of Puccinia glumarum at Cambridge, England, so that during the winter and spring of 1922 no rust could be found. Life is only possible within certain temperature limits and growth has even a narrower range. Moisture may also affect the overwintering of organisms. Many bacteria are killed instantly by ordinary air drying and the fungi vary greatly in their moisture requirements. Humidity affects the survival of many fungous spores. Light may also be related directly to disease producing organisms. Most bacteria are killed by intense sunlight and certain fungous spores may be killed by strong sunlight. Other factors such as the rapid change from one extreme to another, as intermittent freezing and thawing may also be of great importance. In nature, the interaction of all these factors determines whether a given pathogene will survive.

Purpose of the Investigation.

In this investigation an attempt has been made to determine the effect of various factors which may influence the overwintering of the following wheat pathogenes. These are classified according to the diseases caused by them.

A. Foot- and root-rotting fungi.
  1. *Fusarium graminearum* Schwabe
  2. *Helminthosporium sativum* P. K. & B.
  3. *Leptosphaeria herpotrichoides* De Not
  4. *Ophiobolus graminis* Sacc.
  5. *Wojnowicia graminis* (McAlp.) Sacc. et D. Sacc.

B. Black chaff bacterium.
  1. *Bacterium translucens undulosum* S. J. et R.

C. Powdery mildew fungus.
  1. *Erysiphe graminis* D.C.

D. Smut fungi.
  1. *Tilletia tritici* (Bjerk) Wint.
  2. *Tilletia laevis* Kühn

OVERWINTERING OF FOOT- AND ROOT-ROT PATHOGENES.

Until recent years foot- and root-rot diseases of wheat had received little attention in western Canada. However, since it has been realized that they are among the worst enemies of the wheat plant, more attention has been focussed upon them. In the Edmonton district of Alberta they are especially destructive in favorable years for their development, as for instance in 1927 and 1928. Since the winters of this district are usually rather severe it was thought that this would be a particularly favorable location for a study of the overwintering of these parasites.

Literature Review on Overwintering of Foot- and Root-rot Pathogenes of Wheat.

The root-rot and seedling blight of wheat caused by *Fusarium* sp. develops, according to Dickson (9), from two main sources, infected seed and infested soil. He states that the mycelium of the fungus hibernates in or on scabbed kernels of wheat and the organism also develops as a saprophyte on decaying crop refuse near the surface of the soil. At St. Paul, Minnesota, Henry's results (19) indicate that the mycelium and possibly some of the spores of *Fusarium graminearum* and *Fusaria* spp. overwinter on debris in the soil or on the surface. MacInnes and Fogelman (27) demonstrated that the mycelium and spores of *Fusarium graminearum* may live over the winter in Minnesota if protected from the injurious effects of light. Maneval (31) kept ten cultures of *Fusaria* at 10° C. in a refrigerator and they lived from five to eight years. Eight of the cultures were viable after eight years. Atanasoff (2) states that species of *Fusarium* are remarkably resistant to desiccation and low temperatures, and it is supposed that they overwinter in winter crops as mycelium, conidia and chlamydospores. Bennett (3) states that both *Fusarium culmorum* and *F. avenaceum* overwinter in diseased material in the granary and in the field.

Helminthosporium sativum is a widespread and destructive pathogene. The initial infection in the field comes from inoculum borne by the seed, crop residues, or the soil. Christensen (4) states that the mycelium in the seed has been shown to remain viable for several years and that spores kept overwinter outdoors in moist sand did not germinate in the spring. Henry (19) however, found that a rather high percentage of the conidia overwintered on debris in the soil or on its surface. Christensen's (5) later results substantiate Henry's and show clearly that spores of *H. sativum* overwinter under varying conditions in the vicinity of St. Paul. He states that the fungus and spores overwinter in the field on old straw, roots, seed and on the remains of grasses. He also found that there was a great variation in the percentage of spores that remained viable after being buried at different depths in the soil. At Madison, Wisconsin, McKinney (29) showed that the organism survives the winter in the soil and will produce infection on wheat plants in the spring.

Leptosphaeria herpotrichoides is one of the major foot-rotting organisms of Europe and has been found in Alberta, recently, by Henry and Foster (20). In France according to Guyot (16) a mild damp winter favors an attack by the pathogene. He states also that a general attack of foot-rot is favored by high temperatures and excessive precipitation during the preceding winter. Foëx (12) observed that spores were set free from the middle of August until winter and from the

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[illegible]

advent of spring until May the following year. Ascospores were thus found providing material for infection of both winter and spring wheat.

Ophiobolus graminis causes the disease known as "Take-all" which is a destructive foot-rot of wheat in Alberta. Both Kirby (25) of New York and Davis (7) of Wisconsin report that ascospores and mycelium live through the winter. McKinney (29) states that many workers seem to consider that O. graminis infects chiefly, if not entirely, by means of its ascospores, but no doubt the mycelium is of importance also especially as Kirby (25) and Walters (37) have shown that the mycelium can live saprophytically for a time at least. Guyot (16) states that mild damp winters favor an attack in the spring. Kirby (25) found that winter wheat was damaged most and considered that the organism lived over the winter on infected wheat plants.

EXPERIMENTAL RESULTS.

Overwintering in the Soil.

In these, as in the other studies of the foot- and root-rotting fungi, the following organisms were used:

Organism	Spore forms produced	Source
Fusarium graminearum	Conidia	Edmonton
Helminthosporium sativum	Conidia	Edmonton
Leptosphaeria herpotrichoides	Ascospores	Camrose
Ophiobolus graminis	Ascospores	Camrose
Wojnowicia graminis	Pycnidiospores	Edmonton

They were in most cases subjected to the same conditions, so can be directly compared. Monosporous cultures were grown on moist sterilized barley seeds, and this inoculum was added to wooden boxes of soil 1' x 1' x 1'. The boxes were then placed outside in a pit, so that the surface of the soil in the boxes was level with that of the surrounding soil. Soil was then filled in around the boxes.

Effect of soil type. Six different types of soil were used in this experiment. These consisted of the four classified types of Alberta soil as differentiated by the Department of Soils, University of Alberta, and in addition sand and clay subsoil. These may briefly be described as follows:

Type 1. Brown soil of the plains region, where the average annual rainfall is 12-14 inches. Average organic matter content about 4 per cent. Obtained from Brooks.

Type 2. Dark brown soil of transition area between the plains and park belt, where the rainfall averages 14-16 inches annually. Average organic matter content about 6 per cent. Obtained from Kingman.

Type 3. Black soil of the park belt, where the average annual rainfall is 16-18 inches. Organic matter averages about 10 per cent. Obtained at Edmonton.

Type 4. Gray soil of the forest areas, where the average annual rainfall is 15-18 inches. Average organic matter content about 10 per cent. Obtained from Cooking Lake district.

Type 5. Medium fine building sand. Obtained at Edmonton.

Type 6. Clay subsoil. Obtained at Edmonton.

The inoculum was placed at a depth of four inches in each of these soils on November 1, 1928. The boxes were then placed outside. On May 1, 1929, the overwintered inoculum was brought to the laboratory. That of the spore forming organisms was tested for spore germination and that of all of the organisms was tested for growth on potato dextrose agar. The spores were tested on the surface of distilled water in Syracuse dishes, while growth tests were made by plating the inoculated barley seeds on potato dextrose agar, after surface sterilizing them for one-half minute in 70 per cent

alcohol. As shown in Table I, all four organisms tested survived equally well in all the classified types of Alberta soil, that is types 1-4 described above. However, the conidia of both *H. sativum* and *F. graminearum* from sand gave a higher and a more vigorous germination than those from any of the other soil types. A corresponding difference was also noticeable in favor of the sand, in the plating tests of these same fungi. These results agree with Christensen's (5) statement that aeration is an important factor in prolonging the life of the conidia of *H. sativum*.

Table I. Effect of exposure outside of cultures of foot-rotting fungi in different types of soil during the winter of 1928-29.

Treatment	Germination of Conidia		Growth on Agar			
	H.sativum	*F.graminearum*	*F.graminearum*	*H.sativum*	*L.herpotrichoides*	*O.graminis*
Soil						
Type 1	Low	Low	Fair	Poor	Fair	Good
Type 2	Low	Low	Fair	Poor	Fair	Good
Type 3	Low	Low	Fair	Poor	Fair	Good
Type 4	Low	Low	Fair	Poor	Fair	Good
Sand	Medium	Medium	Good	Fair	Fair	Good
Clay	Low	Low	Fair	Poor	Fair	Good

Low = 1-10 per cent; Medium = 10-35 per cent; High = 35-100 percent

Effect of holding inoculum at different depths in the soil.

In this experiment Type 3 soil was used. Barley seed cultures

were placed on the surface and at depths of 2", 4", 8" and 12", on November 1, 1928. Viability tests were made as in the previous experiment on May 1, 1929. The results of these are given in Table II.

Table II. Effect of exposure outside, during the winter of 1928-29, of cultures of foot-rotting fungi at different depths, and in packed and unpacked soil.

	Germination of Conidia		Growth on Agar			
Treatment	*H. sativum*	*F. graminearum*	*F. graminearum*	*H. sativum*	*L. herpotrichoides*	*O. graminis*
Depths of inoculum						
Surface	High -	Low	Excellent	Excellent	Good	Good
2" below	Low -	Medium -	Fair	Poor	Fair	Good
4" below	Low -	Low	Fair	Poor	Fair	Good
8" below	Low -	Low	Fair	Poor	Fair	Good
12" below	Low +	Low	Good	Fair	Fair	Good
Firmness						
Packed	Low	Low	Fair	Poor	Fair	Good
Unpacked	Medium	Low	Fair	Fair	Fair	Good

Low = 1-10 percent, Medium = 11-35 percent, High = 36-100 percent.

All of the cultures showed signs of growth. However, *H. sativum*, *F. graminearum* and *L. herpotrichoides* survived better on the surface than below as shown by the growth on the agar. *Ophiobolus* from all depths grew well. In the germination tests which were

made in Syracuse dishes, the conidia of H. sativum kept on the surface of the soil, germinated 40-50 per cent, while those kept below the surface germinated less than 10 per cent and in most cases less than 3 per cent. At the depth of 12", H. sativum and F. graminearum survived better as shown by growth on agar and by a slightly higher germination of conidia than at 2", 4" and 8" below the surface.

Effect of packing the soil. In this experiment Type 3 soil was used. On November 1, 1928, barley seed cultures were placed at a depth of 4" in boxes of this soil. The soil in two of the boxes was packed by tamping with a block of wood while in two others it was left unpacked. Viability tests were made on May 1, 1929. The results are given in Table II. The survival of H. sativum, as shown by the growth on agar, and germination of conidia, was better in unpacked than in packed soil.

Effect of soil moisture. Type 3 soil was again used in this experiment and the barley seed cultures were placed at a depth of 4". The soil moisture in different boxes was adjusted to initial moisture contents of 20, 25 and 30 per cent degrees of soil moisture which correspond to those commonly obtaining in the field at Edmonton in the fall from year to year. Christensen (5), Kirby (25), McKinney (29), Davis (7), Russel (33), Dosdall (10) and others agree that moisture is an important factor in the development and survival of foot-rot fungi. The results are given in Table III.

Table III. Effect of exposure outside, during the winter of 1928-29 of cultures of foot-rotting fungi in soil of different moisture contents, and in soil of different reactions.

Treatment	Germination of conidia		Growth on agar			
	H. sativum	*F. graminearum*	*F. graminearum*	*H. sativum*	*L. herpotrichoides*	*O. graminis*
Moistures %						
Low 20	Low +	Low	Fair	Poor	Fair	Good
Medium 25	Low	Low	Fair	Poor	Fair	Good
High 30	Low +	Low	Fair	Poor	Fair	Good
Soil reaction						
Acid	Low	Low	Good	Poor	Fair	Good
Neutral	Low	Low	Fair	Poor	Fair	Good
Alkaline	Low	Low	Good	Poor	Fair	Good

In these studies variations in soil moisture from 20 to 30 per cent do not appear to affect the viability or growth of the organisms. It should be noted, however, that these moisture contents were not maintained after exposure outside.

Effect of soil reaction. Three degrees of soil acidity were tested, namely, acid, neutral and alkaline. Type 3 soil was used as the neutral soil and this was mixed with a very acid soil for the acid series and with a very alkaline soil for the alkaline series. Barley seed cultures were placed at a depth of 4". Kirby (25) observed that an alkaline soil produced more

Take-all than one which was non-alkaline. Christensen (5) observed that root-rot caused by *H. sativum* was always worse in alkali spots than on land immediately adjoining them. The results are given in Table III. They indicate that the soil reaction tested had little or no effect on survival as shown by the viability of conidia of *H. sativum* and *F. graminearum* and growth of *H. sativum*, *L. herpotrichoides* and *O. graminis* on agar. The survival of *F. graminearum* in acid and alkaline soil, as shown by growth on agar, was better than in neutral soil.

Overwintering of Foot-rotting Fungi under Natural Conditions on Crop Residues.

Overwintered wheat stubble from fields badly diseased with foot-rots was collected. Spores of three of the foot-rotting fungi, namely, *Leptosphaeria herpotrichoides*, *Ophiobolus graminis* and *Wojnowicia graminis* were present on some of the collections. These were tested for germination in Van Tieghem cells. Pieces of stubble from the various collections were surface sterilized and plated out on potato dextrose agar to determine the viability of any mycelium present in them. The fungi which developed were checked with monosporous cultures of the various foot-rotting organisms on the same medium.

in [illegible] agar that had [illegible] adjusted,
The results are given in Table III. They indicate
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germination and growth of _. sativus, [illegible]
_. [illegible] on agar. The survival of _. [illegible]
and alkaline soil, as shown by [illegible]
than in neutral soil.

[illegible]

[illegible]

[illegible] wheat stubble from fields badly [illegible]
with foot-rots was collected. Spores of three of the
[illegible], Helminthosporium sativum, [illegible]
graminis and Ophiobolus graminis were present on [illegible]
collections. These were tested for germination in
cells. Pieces of stubble from the various collect[illegible]
[illegible]
determine the viability of any [illegible]
fungi which developed were checked with [illegible]
of the various foot-rotting organisms on the [illegible]

Table IV. Survival of foot-rot fungi under natural conditions on wheat stubble during the winter of 1928-29.

Fungus	Isolation of mycelium	Spore germination
Fusarium graminearum	+	- -
Helminthosporium sativum	+	- -
Leptosphaeria herpotrichoides	+	+
Ophiobolus graminis	+	-
Wojnowicia graminis	+	+

+ = spores and mycelium viable.
- = no spores viable.
- - = no tests made.

The mycelium of all organisms mentioned in the table survived the winter on wheat stubble. A few of the ascospores of _L. herpotrichoides_ and _W. graminis_ were viable in the spring, but positive results with the ascospores of _O. graminis_ were not obtained. However, there was not a sufficient number of representative collections of _O. graminis_ ascospores tested to be sure that these spores do not over-winter.

Factors Affecting Overwintering of Foot-rot Fungi.

Effect of low temperatures - continuous and alternating.

The fact that the various foot-rotting fungi survived the severe winter of 1928-29, as has been demonstrated in the previous

experiments, indicates that they are very resistant to low temperatures. The minimum temperature for 1928-29, as shown in Figure I, was -49° F.

In the winter, organisms are exposed to wide variations of temperature both above and below the freezing point. According to Lambert periods of alternating freezing and thawing are apparently a limiting factor in the overwintering of urediniospores of *Puccinia graminis tritici*. Christensen (5) states that a suspension of spores of *H. sativum* in water can withstand alternating freezing and thawing for a considerable time.

In this investigation, barley seeds inoculated with foot-rot pathogenes were kept frozen in ice outside and in the frigidaire. Each morning half of the outside cultures were taken in and allowed to melt at room temperature. As soon as the ice was melted they were put out to freeze again. The remainder of the cultures were kept frozen and tested at varying periods. The results are shown in Tables V and VI.

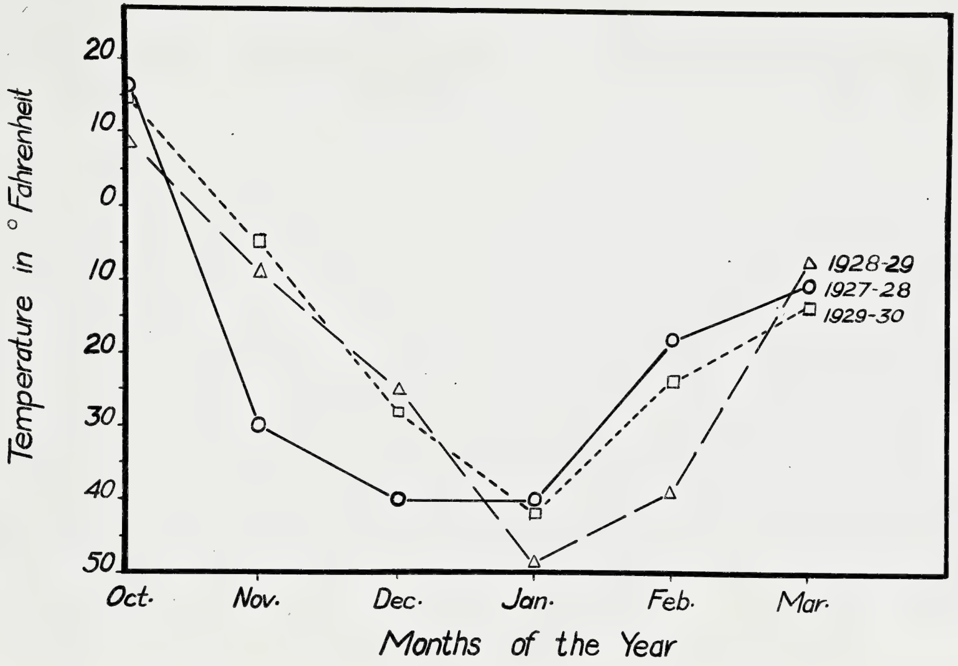

Figure I. Minimum monthly temperatures at Edmonton, Alta., 1927-30.

Table V. Effect of continuous freezing of foot-rotting fungi growing on sterilized barley seeds.

Organism	Source	1 Month		2 Months		3 Months	
		Germ. Conidia	Growth Myc.	Germ. Conidia	Growth Myc.	Germ. Conidia	Growth Myc.
F. graminearum	Edmonton	Med.	+	0	+	0	+
F. graminearum	St. Paul	Med.	+	0	+	0	+
F. graminearum	Baton Rouge	Med.	+	0	+	0	+
H. sativum	Ft.Vermilion	High	+	Low -	+	0	+
H. sativum	Edmonton	High	+	Low -	+	0	+
H. sativum	St. Paul	High	+	Low -	+	0	+
H. sativum	Baton Rouge	High	+	Low -	+	0	+
L. herpotrichoides	Camrose	- -	+	- -	+	- -	+
O. graminis	Camrose	- -	+	- -	+	- -	+
W. graminis	Edmonton	- -	+	- -	+	- -	+

Low = 1-10 per cent, Medium = 11-35 per cent, High = 36-100 percent

Table VI. Effect of continuous and intermittent freezing of foot-rot fungi growing on sterilized barley seeds.

Periods of testing	Number of times frozen and thawed	Germination of *H. sativum* conidia			
		Continuous freezing		Intermittent freezing	
		Germ. of conidia	Veg. growth	Germ. of conidia	Veg. growth
1 day	1	High	+	High	+
3 days	3	High	+	High	+
1 wk.	6	High	+	High	+
2 wks.	12	High	+	Low	+
3 wks.	18	High	+	Low	+
4 wks.	24	Med.	+	0	+
6 wks.	36	Low	+	0	+
8 wks.	40	0	+	0	+

Intermittent freezing was more effective than continuous freezing in killing the spores of H. sativum. Continuous freezing killed one hundred per cent of the conidia in two months while alternating freezing and thawing killed them in about three weeks. The mycelium of all these foot-rotting pathogenes survived frozen solid in ice for at least three months and they withstood alternate freezing and thawing for at least forty times during January and February, 1930.

Germ tubes of H. sativum, only a few hours old, frozen in ice in Van Tieghem cells overnight, resumed growth in many cases after the ice melted the next day. The temperature during the night reached a minimum of 6° F. The spores were kept under observation and measured every hour. A large number of spores were observed by the use of eight microscopes and camera lucida drawings were made of some of them. Plate I shows three typical cases. Most of the germinated spores resumed growth by branching, but a few like No. 7 in Plate I showed renewed growth after thawing from the tips of the germ tubes.

Adaptation of the Pathogene.

The ability of an organism to overwinter in northern regions might conceivably be due to the occurrence of specially cold resistant strains in those areas. In order to test this hypothesis for the foot-rot fungi, strains of H. sativum were obtained from a number of points distributed

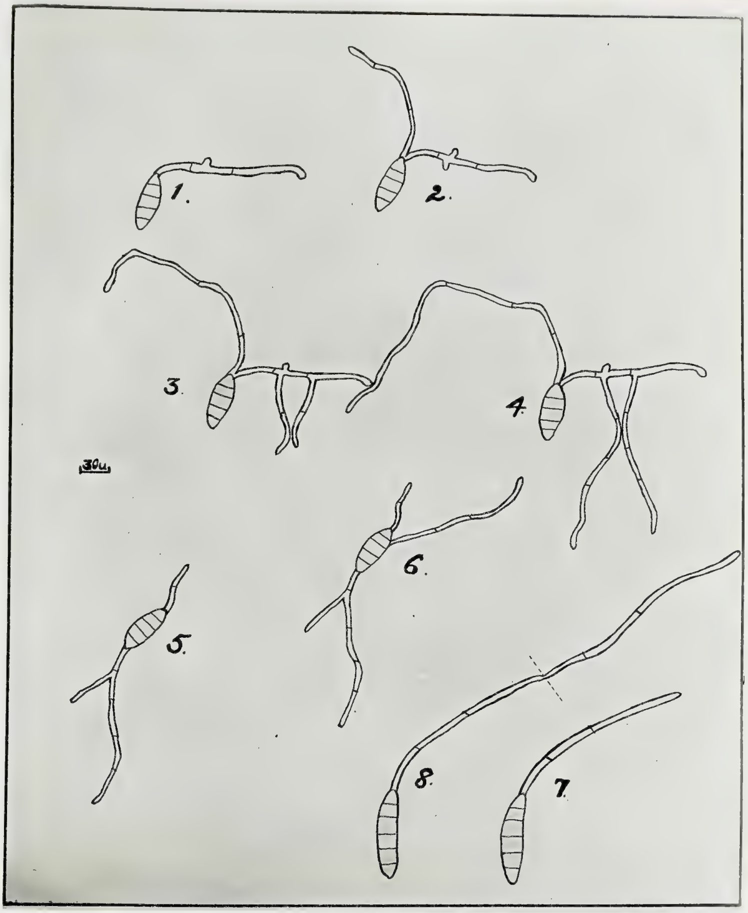

Plate I . Effect of freezing fresh germ-tubes of Helminthosporium sativum in ice, overnight.

1-4. Different stages of one germinated spore before and after freezing. No. 1 shows the stage at which freezing took place; No. 2-4 show stages of growth after thawing.

5-6. No. 5 shows the stage at which another germinated spore was frozen and No. 6 one stage of growth after thawing.

7-8. No. 7 shows the stage at which another germinated spore was frozen and No. 8 shows renewed growth from the tip of the germ tube after thawing.

between Fort Vermilion, Alberta, and Baton Rouge, Louisiana. These cultures were grown on potato dextrose agar and exposed to temperatures ranging from 5 to 35° C. The diameters of the colonies were then measured. A battery of constant temperature incubators illustrated in Plate Ia and wired by the writer proved useful in these experiments.

Table VII. Reaction of different strains of *Helminthosporium sativum* to different temperatures.

Source of strains of *H. sativum*	Diameter of colonies in millimeters at various temperatures						
	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
Fort Vermilion, Alberta	11	15	47	62	88	62	25
Berwyn, Alberta	11	18	32	41	48	76	14
Edmonton, Alberta	11	18	23	49	85	86	25
Ohaton, Alberta	12	15	54	71	88	86	26
Calgary, Alberta	16	23	46	69	88	77	18
Brooks, Alberta	17	25	32	46	72	65	15
Claresholm, Alberta	14	25	45	61	87	60	15
St. Paul, Minnesota	14	18	37	64	75	76	16
Baton Rouge, Louisiana	8	13	30	46	52	58	0

The figures do not indicate that the northern strains grow better at lower temperatures than do those from points farther south. The response of the different strains to temperature seems to be about the same. Berwyn and Baton Rouge strains appear to have a slightly higher optimum than the others.

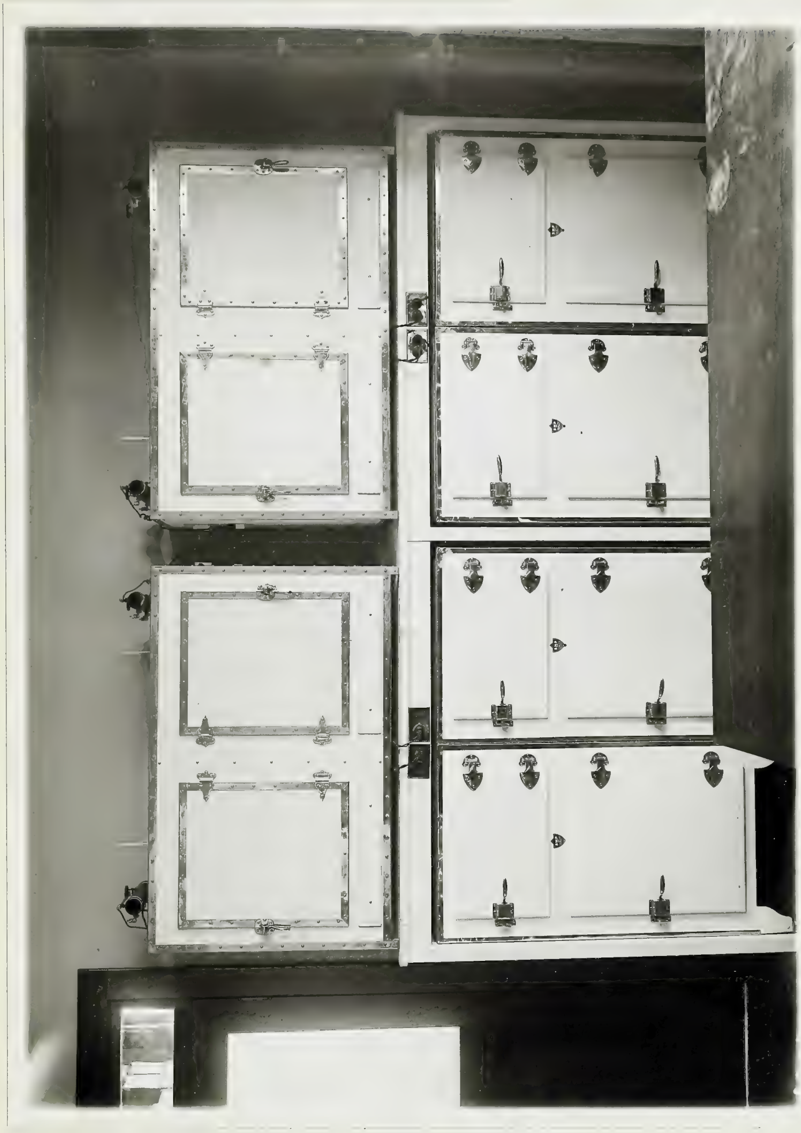

Plate Ia. Controlled temperature incubators.

Similar studies were made with three strains of *Fusarium graminearum*, (Table VIII), of known pathogenicity. These were isolated from wheat heads showing typical scab symptoms. It was thought that the Edmonton strains might be adapted to lower temperatures than the other strains.

Table VIII. Reaction of strains of *Fusarium graminearum* from Edmonton, St. Paul and Baton Rouge to different temperatures.

Source of strains of *F. graminearum*	Diameter of colonies in millimeters at various temperatures.						
	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
Edmonton, Alberta	18	28	60	78	90	84	9
St. Paul, Minnesota	8	19	30	42	72	68	6
Baton Rouge, Louisiana	13	16	26	42	47	37	5

The results show that the Edmonton strain grows more rapidly at all temperatures than the St. Paul and Baton Rouge strains, but in general the reactions of the three strains to different temperatures are similar.

The effect of low temperature on the strains of *H. sativum* and *F. graminearum* following exposure to different humidities and temperature was also studied. The experiment is described on page 31. The results are given in Tables XI and XII. Table XI indicates that the northern strains of *H. sativum* do not survive better than those from points farther south. The most northerly strain from Ft. Vermilion and the most southerly strain from Baton Rouge survived better than the intermediate ones. As shown

in Table XII, the northern strain of F. graminearum from Edmonton survived better than the St. Paul or Baton Rouge strains.

The tendency of an organism to produce new strains by mutations or hybridization might favor its survival during the winter, as some of the new strains might prove more hardy than the parental strain. An illustration of the possibilities in this is afforded by Ophiobolus graminis. During the study of the reactions of the five wheat foot-rotting fungi to temperature, Table IX, it was noted that mutations occurred frequently in Ophiobolus graminis, (Plate IIa), at and near the optimum temperature for its vegetative growth. This agrees with Christensen's results (6) with H. sativum.

Table IX. Reaction of five wheat foot-rotting fungi to temperature.

Organism	Diameters of colonies in millimeters at various temperatures.						
	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
F. graminearum	18	28	60	78	90	84	9
H. sativum	14	16	33	40	90	79	31
O. graminis	21	26	51	67	76	40	7
L. herpotrichoides	10	19	28	35	58	20	0
W. graminis	12	21	24	39	66	25	0

One mutant was selected and compared with its parent, Plate IIb. Cultures of these were grown on potato dextrose

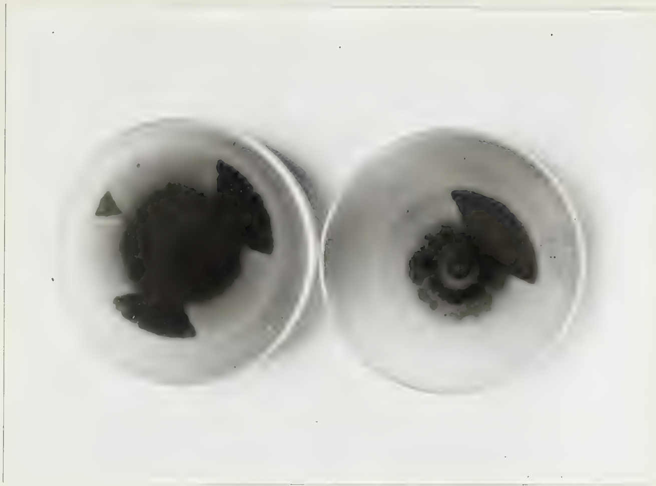

Plate II.a. Mutations in Ophiobolus graminis, obtained on potato dextrose agar at 25° C.

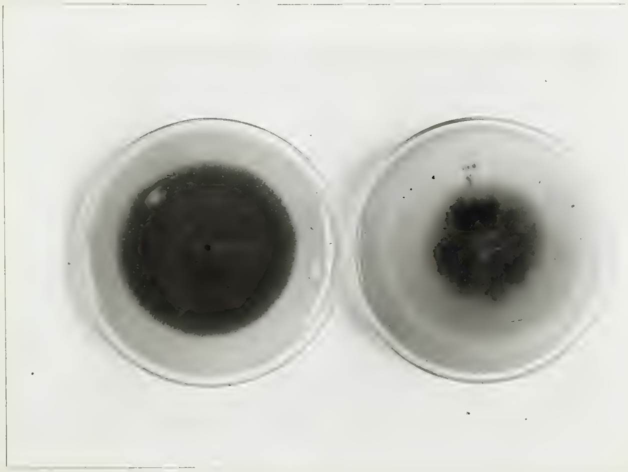

Plate II.b. Colonies of parent (left) and mutant (right) of Ophiobolus graminis on potato dextrose agar at 25° C.

agar and exposed to temperatures ranging from 5 to 35°C. The diameters of the colonies were then measured.

Table X. Reaction of parent and mutant of Ophiobolus graminis to temperature.

Cultures of	Diameters of colonies in millimeters at various temperatures.						
	5°C.	10°C.	15°C.	20°C.	25°C.	30°C.	35°C.
Parent	15	28	46	71	77	59	0
Mutant	24	31	47	71	75	58	0

The results as shown in Table X indicate that the mutant grows better at lower temperatures than the parent, Plate IIIa. Another interesting temperature relation was observed and photographed, Plate IIIb. At low temperatures the parent developed a black pigment a day or more before the mutant but at the high temperature of 30°C. both of them formed black pigments at about the same time.

Condition of the Pathogene.

The winter survival of a fungus may be imperilled by the weather conditions of the previous summer and autumn, which may weaken it or prevent the formation of the spores or other resistant structures by which it normally overwinters. This might well be illustrated by the prevalence of Ophiobolus graminis in Alberta in 1927, 1928 and 1929. In 1927 and 1928,

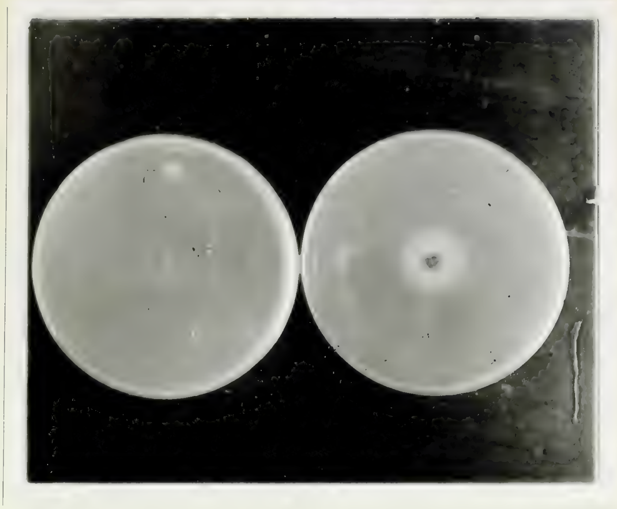

Plate III.a. Colonies of parent (left) and mutant (right) of *Ophiobolus graminis* on potato dextrose agar at 5° C.

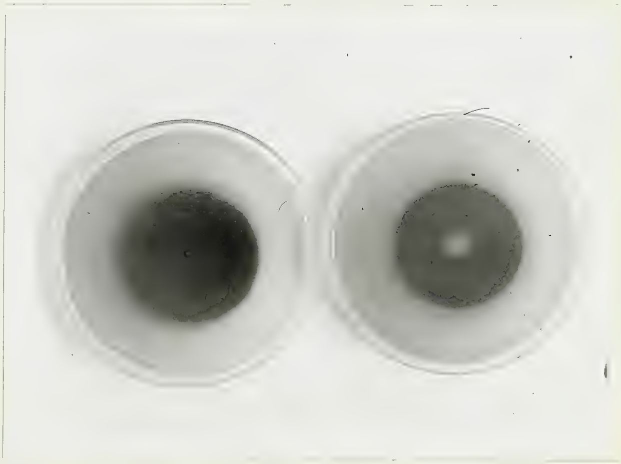

Plate III.b. Colonies of parent (left) and mutant (right) of *Ophiobolus graminis* on potato dextrose agar at 30° C.

environmental conditions must have been favorable for the development of this organism because the symptoms caused by it were plentiful and considerable damage occurred. However, in 1929, conditions must have been unfavorable because the typical symptoms were difficult to find. An examination of Figure II shows that temperature conditions differed very little in the summer months of the three years in question. On the other hand, Figure III shows that the precipitation for 1929 was much lower, especially for May, June and July. This supports Kirby (25), McKinney (29), Davis (7) and Russel (33) in their contention that wet soils favor the development of the Take-all fungus. Thus lack of moisture may be the limiting factor which may weaken or prevent the formation of the spores and mycelium by which the fungus is normally able to overwinter.

It is obvious that the condition in which an organism goes into the winter will affect its survival. The previous history, the environmental conditions of the summer and fall may have an effect on the amount and hardiness of the inoculum. It was thought that it would be advisable to study the effect of exposing the different foot-rotting fungi to variable environmental conditions before subjecting them to freezing temperatures. In this investigation, the effect of low temperature on the five foot-rot fungi and strains of _H. sativum_ and _F. graminearum_ was studied following their exposure to different humidities and temperatures.

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typical symptoms were difficult to find. As exampl
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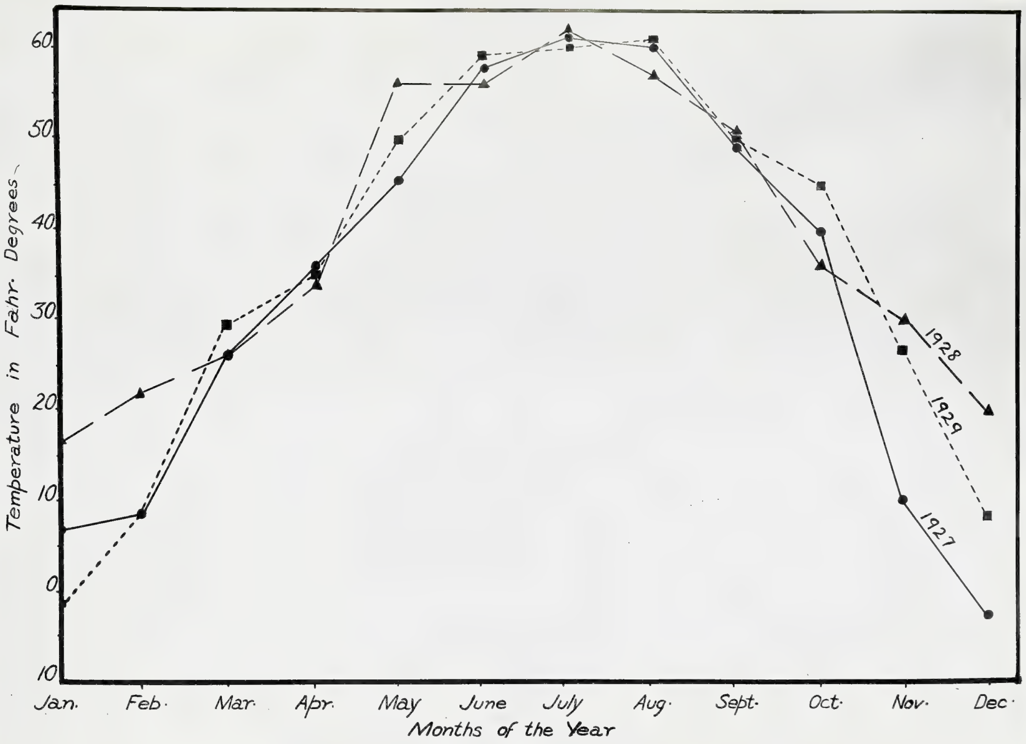

Figure II. Mean monthly temperatures at Edmonton, Alta., 1927-29.

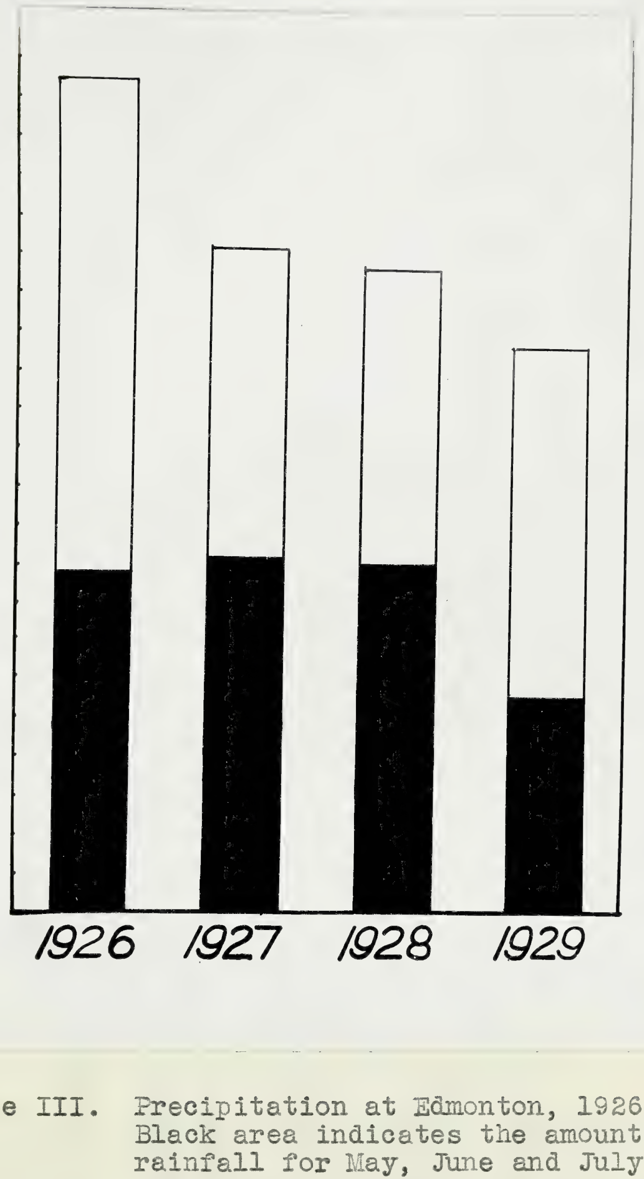

Figure III. Precipitation at Edmonton, 1926-29. Black area indicates the amount of rainfall for May, June and July. Each division represents one inch of rainfall.

Spaulding and Gravatt (36), Hart (17), Christensen (5) and many others have shown that humidity affects the survival of fungous spores. Christensen (5) states that the conidia of H. *sativum* can withstand exposure to low temperatures for considerable periods if the relative humidity is low.

Cover slides and wheat hulls coated with acid agar were placed on petri dish cultures until they were overgrown with mycelium. The slides were then placed in test tubes containing the following saturated solutions:

Saturated Solution	Humidity %
Water, distilled H_2O	100
Sodium chloride $NaCl$	73
Copper nitrate $Cu(NO_3)_2$	45
Calcium chloride $CaCl_2$	26
Sulphuric acid H_2SO_4 Sp. gr. 1.84	0

The test tubes were then sealed with non-absorbent cotton and paraffin and placed in the constant temperature chambers from 5° to 35°C. for varying periods of time. The cultures were then subjected to -5°C. also for varying periods. The results are given in Tables XI, XII and XIII.

In these experiments, the cultures marked as having been killed were, according to viability test, dead before exposure to -5°C. The cultures kept at the high temperature of 35°C. and at high humidities were the first to die.

Table XI. Effect of low temperature on *F. graminearum* and six strains of *H. sativum* following their exposure to different humidities and temperatures.

	Temperature °C.	5° C.					15° C.					25° C.					35° C.				
	Humidity %	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100
Organism	Place																				
	Exposed for 1 day before freezing																				
F. graminearum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	Ft.Vermilion, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	Berwyn, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	Calgary, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	St. Paul, Minn.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H. sativum	Baton Rouge, Louis.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Exposed for three weeks before freezing																				
F. graminearum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	Ft.Vermilion, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
H. sativum	Berwyn, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	Calgary, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	St. Paul, Minn.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	Baton Rouge, Louis.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Table XII. Effect of low temperature on *L. herpotrichoides*, *O. graminis*, *W. graminis* and three strains of *F. graminearum* following their exposure to different humidities and temperatures.

	Temperature °C.	5° C.					15° C.					25° C.					35° C.				
	Humidity %	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100
Organism	Place																				
	Exposed for 1 and 2 weeks before freezing																				
F. graminearum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
F. graminearum	St. Paul, Minn.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
F. graminearum	Baton Rouge, Louis.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
L. herpotrichoides	Camrose, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
O. graminis	Camrose, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
W. graminis.	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	Exposed for 3 months before freezing																				
F. graminearum	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
F. graminearum	St. Paul, Minn.	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
F. graminearum	Baton Rouge, Louis.	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
L. herpotrichoides	Camrose, Alta.	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
O. graminis	Camrose, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
W. graminis	Edmonton, Alta.	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

Table XIII. Effect of low temperature on F. graminearum, H. sativum and L. herpotrichoides following their exposure to different humidities and temperatures.

Exposed for 1 month before freezing

Temperature °C.	5°C.					15°C.					25°C.					35°C.				
Humidity %	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100	0	26	46	73	100
Organisms																				
F. graminearum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
H. sativum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
L. herpotrichoides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

All of the cultures kept at 5° to 25°C. and at relative humidities of 0 to 100 per cent for one month survived exposures of -5°C. At the end of three months all the culture kept at 35°C. were dead, and at 25°C. the cultures were dead at the high humidities. The remaining cultures kept for three months all survived. Table XIII indicates that the relative resistance of the following organisms are arranged in their ability to survive as follows: H. sativum, F. graminearum and L. herpotrichoides.

OVERWINTERING OF BACTERIUM TRANSLUCENS VAR. UNDULOSUM

The above organism causes a bacterial disease of wheat known as Black Chaff, a disease which is now quite prevalent in Alberta. This disease was first described by Smith (34) in the United States. Little further pathological work has been published on Black Chaff. Smith states that Bacterium translucens var. undulosum is remarkably resistant to sunlight and dry air, remaining alive and infectious on dry seed for a year. Nothing, however, is said regarding the overwintering of the organism in the open.

In this investigation an attempt was made to learn something of the conditions under which this bacterium may survive the winter at Edmonton. On November 1st, 1929, inoculum consisting of heavily infected heads and culms of Marquis wheat was deposited at different depths in the soil in wooden boxes which were buried to soil level. The inoculum on the surface was examined every month and plated out on potato dextrose agar. Data on the inoculum kept below the soil surface will not be taken until May 1, 1930.

Table XIV. Viability tests of Bacterium translucens var. undulosum kept on the surface of the soil at Edmonton in naturally infected heads and culms of wheat from Nov. 1, 1929, to April 1, 1930.

Date	Viability of the Bacterium
Nov. 1	+
Dec. 1	+
Jan. 2	+
Feb. 1	+
March 1	+
April 1	+

+ = typical colonies of Bacterium translucens undulosum recovered.

As indicated by Table XIV B. translucens undulosum remained alive throughout the winter at least until April 1, in dead wheat tissues kept on the surface of the soil. Recovered colonies were compared each time with colonies of the organism isolated from check material kept inside and appeared identical in all cases.

Another experiment was made to determine the effect of continuous and intermittent freezing on the viability of this organism. The results are presented in Table XV.

Table XV. Effect of continuous and intermittent freezing on the survival of B. translucens undulosum in dead wheat tissue.

	No. of freezings and thawings	Method of freezing	
		Continuous	Intermittent
1 day	1	+	+
3 days	3	+	+
1 wk.	6	+	+
2 wks.	12	+	+
3 wks.	18	+	+
4 wks.	24	+	+
6 wks.	36	+	+
8 wks.	40	+	+

It is clear from the above table that the Black Chaff bacterium survived two months of continuous freezing, and alternating freezing and thawing for at least forty times during January and February of 1930.

OVERWINTERING OF *ERYSIPHE GRAMINIS*.

Erysiphe graminis occurs quite commonly in Alberta especially on winter wheat, but its method of overwintering here is not definitely known.

In summarizing the methods of overwintering of powdery mildew fungi Heald (18) states that,

> "the perithecia in most cases are overwintering fruits, the ascospores being able to germinate after being exposed to winter temperatures. In a few species the ascospores may be ejected in the fall under favorable conditions of moisture and temperature and germinate at once, but the general period for ascospore expulsion is during the warm spring rains.----- It is known that some powdery mildews, especially certain species attacking woody hosts, can survive without the production of perithecia.--- Even in certain species in which the perithecia are commonly formed, it is the belief that they play a minor part in carrying the fungus over the winter. In such cases the dormant mycelium either hibernates in the winter buds and resumes activity when buds open in the spring, or special cells are formed which are highly resistant."

The conidia are normally short lived and are not ordinarily able to survive the winter.

In order to determine how *Erysiphe graminis* overwinters at Edmonton, conidia, mycelium and perithecia were examined periodically. The conidia were not only very difficult to find late in the fall, but did not germinate on or after November the 1st as shown in Table XVI.

Table XVI. Viability tests of conidia of *Erysiphe graminis* from collections made in the field at Edmonton.

Date	Germination of conidia
	%
Oct. 1	11
Nov. 1	0
Dec. 1	0

The viability of the mycelium was tested by bringing in from the field numerous leaves of winter wheat, many of which were infected in the fall, and keeping them for several days on moist filter papers in petri dishes. Examination of a large number of leaves as shown in Table XVII indicated that mycelium did not grow and develop conidia. Furthermore, powdery mildew did not appear on winter wheat until June 11th, in 1928 and June 15th, 1929, which indicates that the mycelium does not live over the winter.

Table XVII. Viability tests of mycelium of *Erysiphe graminis* from collections made in the field at Edmonton.

Date	Growth on leaves
Oct. 1	-
Dec. 1	-
Jan. 1	-
Feb. 1	-
Mar. 1	-
May 1	-
June 1	-

- = no growth

Perithecia were brought in from the field at various times throughout the fall and winter, crushed and examined under the microscope. No differentiation of the asci occurred during this period. About the middle of May, however, the asci began to differentiate and by the middle of June numerous mature ascospores were found (Plate IV). In the spring, infection was first observed on wheat nearest the winter wheat plot bearing the overwintered perithecia. It appears from the observations made that Erysiphe graminis overwinters at Edmonton, only in the perfect stage.

Table XVIII. Development of ascospores of Erysiphe graminis in the field at Edmonton.

Date	Ascospores formed
Oct. 1	-
Nov. 1	-
Jan. 3	-
Feb. 4	-
March 1	-
April 1	-
May 1	-
May 15	+
June 1	+
June 8	++
June 15	++

- = no ascospores differentiated
+ = ascospores beginning to differentiate
++ = ascospores differentiated.

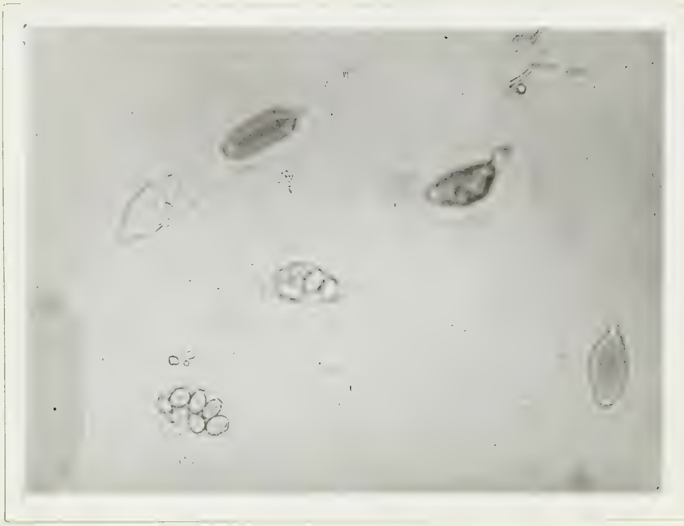

Plate IV. Differentiation of ascospores in asci of Erysiphe graminis.

Lower left-hand corner; ascus with eight ascospores, showing the degree of differentiation found in late May following rain.

Lower right-hand corner; undifferentiated ascus, the type found in the fall and throughout the winter.

OVERWINTERING OF TILLETIA TRITICI AND TILLETIA LAEVIS

Bunt or Stinking Smut of wheat in Alberta may be caused by either Tilletia tritici or Tilletia laevis, but the former species is the more common. The disease was unusually prevalent in western Canada in 1929 especially on winter wheat.

The bunt fungi are known to commonly overwinter in western Canada in the form of chlamydospores adhering to the surface of the seed. Experimental evidence, however, is lacking to prove whether soil-borne chlamydospores overwinter in western Canada. Woolman and Humphrey (38) have demonstrated in the Palouse region of Idaho and Washington Oregon and California, that soil-borne spores may cause a smutty crop of winter wheat even though the seed is carefully treated. However, when spring wheat is sown on similarly infested soil the crop remains practically bunt free, indicating that the spores do not survive the winter in the soil in that region. In western Canada, where conditions are considerably different, Güssow and Conners (15) state that as a rule threshing is done so late in the fall that the soil is too cold to permit the germination of any spores which are soil borne; and that these loose spores as well as the spores in smutted heads and unbroken bunt-balls which have fallen to the ground during harvesting, may remain viable throughout the winter and cause infection the following year. They further state, however, that the effectiveness of seed treatments in

[illegible]

by either *Tilletia tritici* or [illegible]

[illegible]

prevalent in western [illegible]

wheat.

The bunt fungi are known to [illegible] overwinter

western. Dunce in the [illegible]

surface of the soil. [illegible], however,

[illegible] to prove [illegible] soil-borne spores

in western [illegible] (11), have

demonstrated in the [illegible] region of Idaho and [illegible]

Oregon and California, that soil-borne spores may [illegible]

smutty crop of winter wheat even though the seed is

treated. However, when spring wheat is sown on [illegible]

[illegible]

indicating that the spores do not survive the winter

soil in that region. In western Canada, where [illegible]

considerably different, [illegible] and [illegible] (

as a rule threshing is done so late in the fall that

is too cold to permit the germination of any spores

soil borne; and that these local sources as well as

[illegible]

[illegible], [illegible] results visible [illegible]

[illegible] following year. The

controlling bunt indicates that infection from spores which have overwintered does not commonly occur.

Bunt spores may germinate in the soil and infection of nearby seedlings may take place if conditions are suitable. The spores germinate at temperatures as low as 5°C. and as high as 25°C. Hungerford (23), Gibs (14), Güssow and Conners (15), Heuser (21) and others agree that the optimum temperature for infection is about 9° to 12°C. Faris (11) states that temperatures ranging from 5° to 15°C. are highly favorable for infection by either species. Woolman and Humphrey in 1914 obtained over 80 per cent infection at the low temperature of 3.6°C. Moisture also bears an important relation to the germination and survival of bunt spores. Hungerford's work (23) indicates that bunt spores present in the soil in the Pacific Northwest from a previous year will not remain viable till the following spring regardless of moisture. Foister (13) quotes Mackie to the effect that bunt spores need 14 per cent soil moisture in order to germinate. Woolman and Humphrey (38), Appel and Riehm (1) and others state that overwintering of free spores does not occur.

Experimental Results.

On September 6th, 1928, bunt spores were distributed both in rows and broadcast in field plots at Edmonton. Disinfected Kharkov winter wheat was seeded in half of the plot and on April 26th, 1929, the remaining half was sown to Marquis wheat.

Temperature and moisture conditions were highly favorable for infection both in the fall and spring. When the grain was nearly mature, the percentage of bunt was recorded and specimens were collected to determine the species that overwintered.

Table XIX. Effect of soil infestation with bunt spores on the development of bunt in winter and spring wheat.

Seed Treatment	Soil treatment	Winter wheat Per cent heads infected	Spring wheat Percent heads infected
Check	No inoculum	0.0	0.0
Untreated	inoculum added to rows	14.0	0.0
Copper Carbonate	inoculum added to rows	9.7	0.0
Formalin	inoculum added to rows	10.0	0.0
Hot water	inoculum added to rows	10.0	0.0
Check	No inoculum	0.0	0.0
Untreated	inoculum broadcasted	13.5	0.0
Copper Carbonate	inoculum broadcasted	8.5	0.0
Formalin	inoculum broadcasted	10.7	0.0
Hot water	inoculum broadcasted	6.0	0.0

From Table XIX it will be noted that winter wheat became infected with bunt when sown in soil infested with bunt spores while spring wheat did not. Soil-borne spores of both Tilletia tritici and Tilletia laevis caused infection of

winter wheat. The bunt spores evidently did not survive the winter in the soil, but the mycelium in winter wheat plants readily overwintered. It is also of interest to note that the infection of winter wheat was practically as high on the plots where the spores were broadcast as on those in which the spores were deposited in the rows where the seed was sown. There seems, therefore, to be a possibility of winter wheat becoming infected with bunt in Alberta from soil-borne spores. Seed treatment as shown by Table XIX will not prevent this type of infection. The greater prevalence of bunt in winter wheat in Canada as compared with spring wheat may be partly accounted for by the above results. According to estimates of the Board of Grain Commissioners of Canada there was proportionally seventy, fifty and eighteen times as much bunt in winter wheat in 1926, 1927 and 1928 respectively, in Canada, as in spring wheat. Furthermore, contrary to statements of Güssow and Conners, fall conditions at threshing time are often favorable for infection of winter wheat. Thus the mean monthly air temperature for September at Edmonton for the last forty-seven years was 50°F. and according to Güssow and Conners maximum bunt infection occurs at 46-50°F. Figure IV shows the soil temperature 2" below the surface for the last three weeks in September 1928 was somewhat higher than the optimum. It appears that temperature conditions in the fall at Edmonton are generally favorable for infection from soil-borne bunt spores.

[illegible]

mostly overall lower. It is also [illegible] fluctuation [illegible] where the spores were [illegible] spores were deposited in the [illegible] Rose seems, therefore, to be [illegible] becoming infected with [illegible] seed treatment [illegible] type of infection. The greater prevalence of [illegible] in [illegible] as compared with [illegible] accounted for by the above mentioned. According to [illegible] of the [illegible] [illegible] in winter wheat [illegible], [illegible] and 1931 respectively, as in earlier [illegible]. [illegible], tendency to [illegible] Tisdale and Johnson, [illegible] conditions [illegible] favorable for infection of winter wheat. Thus the air temperature for September at [illegible] for the [illegible] seven years was 50°F. and according to [illegible] maximum smut infection occurs at [illegible]–50°F. [illegible]

[illegible]

[illegible]

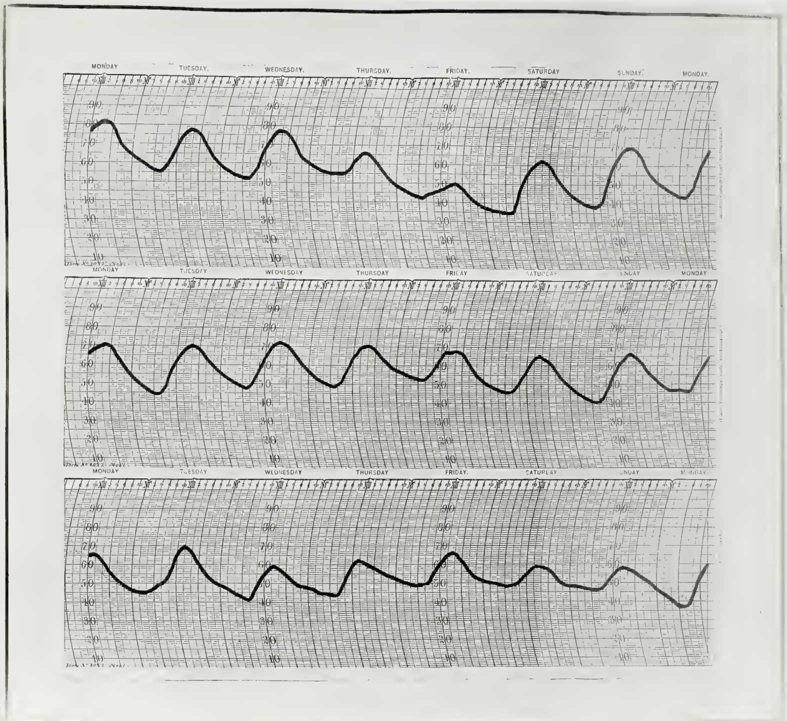

Figure IV. Soil temperatures at Edmonton taken 2 inches below the surface during the last three weeks of September, 1928.

Summary.

1. The foot- and root-rotting pathogenes, *Fusarium graminearum* Schwabe, *Helminthosporium sativum* P. K. and B., *Leptosphaeria herpotrichoides* De Not, *Ophiobolus graminis* Sacc. and *Wojnowicia graminis* McAlp., Sacc. and D. Sacc. survived the severe winter of 1928-29 at Edmonton, Alberta.

2. The mycelium of these five fungi survived the winter on barley seeds and on wheat stubble.

3. Spores of *H. sativum*, *F. graminearum*, *L. herpotrichoides* and *W. graminis* were viable in the spring after overwintering in the open.

4. Four of the foot-rotting fungi survived equally well in all the classified types of Alberta soil.

5. Well aerated soil, such as sand and unpacked soil, favored the survival of the conidia of *H. sativum*.

6. *F. graminearum*, *H. sativum* and *L. herpotrichoides* survived better on the surface of the soil than below. The conidia of *H. sativum* survived much better on the surface than underground.

7. Strains of *H. sativum* and *F. graminearum* from northern latitudes did not grow better at low temperatures than southern strains nor did they show any consistently greater resistance to freezing temperatures.

8. Mutations arose frequently in Ophiobolus graminis at or near the optimum temperature for its vegetative growth 20-25°C. A selected mutant grew better at the low temperature of 5°C. than its parent.

9. Alternating freezing and thawing was more effective in killing the spores of H. sativum than continuous freezing. Continuous freezing killed one hundred per cent of the spores in two months while alternating freezing and thawing killed the spores in three weeks.

10. Mycelium of all these fungi in inoculated barley seed survived, frozen solid, for at least three months. They also survived alternate freezing and thawing for at least forty times during January and February of 1930.

11. Germ tubes of H. sativum only a few hours old survived freezing solid overnight. Renewed growth after freezing was usually branching, but in some cases from the tip.

12. All of the cultures kept at 5° to 25°C. at relative humidities from 0 to 100 per cent for one month survived exposures of different lengths to -5°C.

13. Bacterium translucens undulosum, in plant tissue, survived the winter of 1929-30 at least until April 1.

14. In plant tissue, Bacterium translucens undulosum survived alternate freezing and thawing for at least forty times.

15. The conidia and mycelium of *Erysiphe graminis* D.C. did not appear to overwinter.

16. *Erysiphe graminis* appears to overwinter in the perfect stage at Edmonton, Alta.

17. The bunt fungi, *Tilletia tritici* and *Tilletia laevis* overwintered at Edmonton as mycelium in winter wheat.

18. Soil borne spores of *Tilletia tritici* and *Tilletia laevis* did not survive the winter of 1928-29 at Edmonton.

19. Temperature at threshing time in the fall at Edmonton is favorable for the germination of soil-borne bunt spores.

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